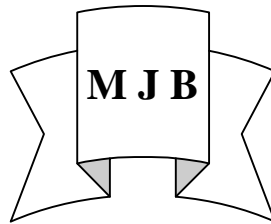


the protection of liver by fenugreek seeds from the effects of prostaglandin $f_2 a$
in male albino mice

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Abstract

Prostaglandins (PGs) have been used for many medical aspects such as parturition , blood pressure control and another . Numerous studies have been published about their effects on the kidney , thyroid gland , male and female reproductive system. But no previous studies has dealt the possibility of minimizing their effect or even repair the damaging structure has been reported. These experiments were designed to study the effect of $PGF_2 a$ on hepatic tissue and then use fenugreek seeds to overcome the $PGF_2 a$ effects on hepatic tissue . We concluded that fenugreek seeds could inhibit the negative side effects of $PGF_2 a$ in the liver especially by using six dose of fenugreek after $PGF_2 a$ injection in similar doses.

الخلاصة

استخدمت الموثينات في العديد من المجالات الطبية كحث الولادة وتنظيم ضغط الدم وغيرها . اجريت العديد من الدراسات حول تأثير الموثينات على الكلية والغدة الدرقية والجهاز التناسلي الذكري والانثوي . ولعدم وجود دراسات سابقة عن امكانية التخلص من تاثيراتها الجانبية تم في انسجة الكبد . $a F_2$ في النسيج الكبدي ثم استعمال بذور الحلبة للتخلص من تاثير الموثين $a F_2$ اجراء هذه الدراسة لمعرفة تأثير الموثين $a F_2$ خاصة عند استعمال ست جرعة من الحلبة بعد الموثين $a F_2$ نستنتج من هذه الدراسة بان لبذور الحلبة تاثير مثبط للتاثير السلبى للموثين بنفس عدد الجرعة.

Introduction:

Prostaglandins (PGs) play diverse and important roles in human health and disease states and regulate abroad range of physiological processes such as pregnancy, labor (

parturition) (1,2,3) , blood pressure control (4) , modulation of inflammation and immune response (5) , liver protection and damage (6). In many malignant tumours including some liver tumours increased

levels of PGs , most notably PGE₂ and PGF₂*a* , have been detected (7). PGs stimulate tumour growth and they presumably act in most of carcinogenesis (8).

Most PGs exert their actions by binding to specific cells surface receptor , PGF₂*a* may act on it's G Protein-Coupled receptor (FP) (9,10,11).Receptors for PGs have been identified in various tissues (12) such as liver (13,14). PGF₂*a* may be imported intracellular via a transporter which has high affinity for PGF₂*a* which increases intracellular cyclic AMP (15) .

Previous studies have been showed some PGs have effect on protein and lipid metabolism (16). Also have effect on carbohydrate metabolism, the studies about effects of PGs on glycogenesis revealed that PGE₂ (10µg/ml) significantly inhibited incorporation of glucose into glycogen pool. PGE₂ at (1-10 µg /ml) stimulated incorporation of glucose into non-glycogen macromolecules and these results indicated that PGE₂ and PGF₂*a* ,in physiological concentration, directly influence the metabolism of glucose (17).Also PGs have inhibitory effect on insulin secretion which in turn effect on carbohydrates , lipids and protein metabolism (18) especially PGF₂*a* at (5µg/Kg body weight) which cause

degenerative changes in beta cells of pancreas including vacuolation , decreasing or losing of secretory granules of insulin and increasing apoptotic beta cells (19).

Fenugreek seeds have been used as a food spice and in traditional medicine for numerous indications including labor induction, aiding digestion and as a general tonic to improve metabolism and health .Many animal and human trials suggest possible hypoglycemic and antihyperlipidemic properties of oral fenugreek seeds powder (20,21).

Present interest in fenugreek focuses on its potential benefits for people with diabetes or high cholesterol . Fenugreek seeds which rich in proteins contain the unique major amino acid 4-hydroxyisoleucine which has been characterized as one of the active ingredients in fenugreek for blood glucose control (22,23).

Materials and Methods:-

Three groups of albino male mice *Mus musculus* (12-18 weeks old) were used in this study . The temperature in animal house was 22-30 C° with a system of (12 hours light :12 hours dark). All mice was given water and pellet *ad libitum* , 20 mice were used and divided into equal groups (five for each) as the following :-

- 1- The first group of animals were injected subcutaneously daily for 6 days with 0.1 ml normal physiological saline (0.9% NaCl) and considered as a control group.
- 2- The second group was injected subcutaneously daily for 6 days with 0.1 ml prostaglandin F_2a ($5\mu\text{g/Kg}$ body weight) . PG_2a (Veteglan) from Spanish Calier company was diluted with sterilized normal physiological saline to preparation the concentration used in this experiments (19).
- 3- The third group was given PGF_2a as the second group then was given watery solution of fenugreek seeds orally with a dose (1.6 mg/gm body weight) daily for 6 days. This group subdivided into two groups:
 - a- First group was given fenugreek after 30 minutes from PGF_2a injection.
 - b- Second group was given six doses of fenugreek after injection with six doses of PGF_2a .

Body weight was taken before and after the experiments then the animals killed by spinal dislocation after 24 hours from the last dose of treatment. The liver was

removed , weighted , fixed in formalin 10% solution , processed and 5 micron thickness paraffin sections were stained with Hematoxylin and Eosin (HE) methods and glycogen stain(Periodic Acid Schiff(PAS))(24) for light microscopic observation and examined. The liver / body weight ratio was calculated as follow:

$$\text{Liver /body weight ratio} = \frac{\text{Liver weight (gram)}}{\text{Body weight (gram)}} * 100$$

The results statistically analyzed by using analysis of variance (ANOVA) and standard error was calculated (25).

Results & Discussion:-

First : liver weight:

(a) Animals group treated with PGF_2a :

There was no significant decrease in liver /body weight ratio in PGF_2a treated group (Table -1-).The cause of decreasing although it was not significant may be due to the degeneration of many hepatocytes or because the PGs inhibited incorporation of glucose into glycogen which showed in this study.Glycogen stained with PAS appear as purple patches (24) was few in this group compared with control group (figure-1-).The decreasing of glycogenesis occur by directly

influence of PGF_2a on the metabolism of glucose (17) through the inhibitory effect on insulin secretion by causing degenerative changes and apoptosis of beta cell (19).

(b) Animals treated with PGF_2a and fenugreek seeds:

There was no significant differences in animals treated with PGF_2a and fenugreek seeds solution. This may be due to administration of PGF_2a decrease the liver weight compared with control group but when treated with fenugreek seeds (especially 6 doses of fenugreek solution after 6 doses PGF_2a injection) enhanced the liver weight because fenugreek seeds increasing insulin secretion and increase glycogenesis (20,21) and then increase glycogen storage in liver as seen in figure-1- which appear abundant and then effect on liver weight. Our results agree with (26) who reveals that fenugreek seeds boiled extract caused a significant increase in liver glycogen levels and liver / body weight percentage .

Second : - Histopathological study:-

a- Animals group treated with PGF_2a :-

Most hepatocytes showed marked degenerative changes in both nucleus & cytoplasm, only few hepatocytes remained

with normal appearance . We can summarized histological changes of follow:-

1- The most characteristic changes in the liver were increase in the size of most hepatocytes and their nucleus (cellular hypertrophy) , while another hepatocytes contain irregular nuclear envelope (figure: 2 A,B) . Hepatic lobules contain many hepatocytes with vacuolated cytoplasm (Figures 2,3,4) .Some Hepatic lobules occasionally had few atrophied cells which reveals to necrosis . The nucleus of each necrotic cell was smaller , condensed and intensely stained with hematoxylin (figure : 2B , 4A). These changes might be due to the presence of PGF_2a receptors on hepatocytes (13,14) or might be due to the effect of PGF_2a on membrane permeability (27) who revealed that gap junction permeability between hepatocyte doublets was strongly inhibited by prolonged (2h) treatment with PGF_2a which may enhance or diminish the propagation of Ca^{2+} signals which is important for certain hepatic functions including biliary flow and glucose output.

2- Some sinusoids were enlarged (figure : 2C,2D,4B) because degeneration of most hepatocytes while another sinusoids were normal.

3- Increasing of Kupffer cells number as seen in (figure :2) may due to activation

of Kupffer cells which have been documented to play an important role in the early events of liver injury and regeneration by releasing biologically active mediators such as Interleukin-6(IL-6) (28).

4-Vascular congestion (figure:3C,D), infiltration of inflammatory cells at periphery of central vein (figure:3) and edema within hepatic lobules was frequently observed as signs of inflammatory process(figure:4, 2D). These results indicate PGF_2a is mediator of inflammation (5) through its role in vasodilatation which first cause increased blood flow then stasis of blood flow and increased capillary permeability then accumulation of fluid into tissues and second neutrophils migrate to site of tissue damage by chemotactic agents from tissue debris (29) .

b- Animals treated with PGF_2a & Fenugreek seeds :

There was histological changes in hepatic tissue of animals group treated with fenugreek seeds after 30 minutes from PGF_2a injection but they were less than in animals group treated with PGF_2a alone . These changes include increased proliferate hepatocytes, some enlarged sinusoids , congestion & infiltration of inflammatory

cell (figure : 5 A,B) but many parts of another lobules had normal architecture (figure : 5C) . While animals group which treated 6 doses of fenugreek seeds after 6 doses of PGF_2a demonstrate hepatic architecture like normal hepatic tissue (figure:5D)

These results may be due to the presence of several compounds in fenugreek seeds or may be because fenugreek seeds increasing the sensitivity of tissue to available insulin (30) .

C- Control group : light microscopic examination of the liver revealed the presence of normal morphology of hepatocytes and normal architecture of liver (figure:6). These finding are similar to those described by other authors (31)

Conclusion:-

The present study conclude that the PGF_2a had negative effect in histological structure of liver and fenugreek seeds could inhibit this negative effect in liver especially by using 6 doses of fenugreek after 6 doses of PGF_2a injection . We advise to use fenugreek seeds in future studies to inhibit PGs & other hepatotoxic drugs side effects by using fenugreek seeds in longer time than 6 days.

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Table -1- Liver / body weight ratio changes in treated groups.

Groups	Liver/ body weight ratio (Mean ± S.E)
Control	5.56 ± 0.76
PGF ₂ a	4.75 ± 0.16
PGF ₂ a + fenugreek seeds	5.20 ± 0.41
PGF ₂ a (6 doses) + fenugreek seeds (6 doses)	5.45 ± 0.07

S.E : Standard Error

N= 5

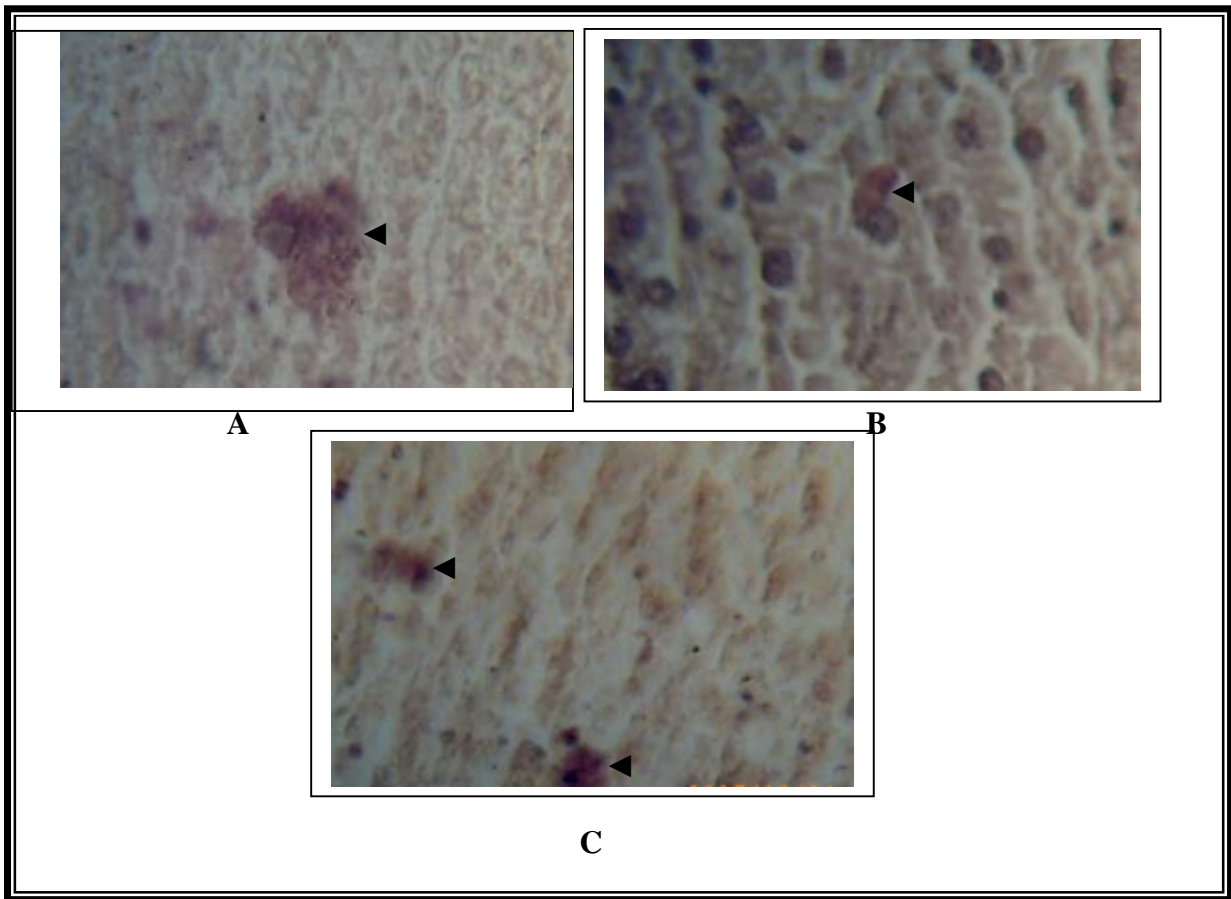


Figure-1:- Hepatic tissue of mice stained with PAS stain showed glycogen (▲)

A: control group

B: PGF₂a treated group

C: Fenugreek & PGF₂a treated group

(PAS: 600X)

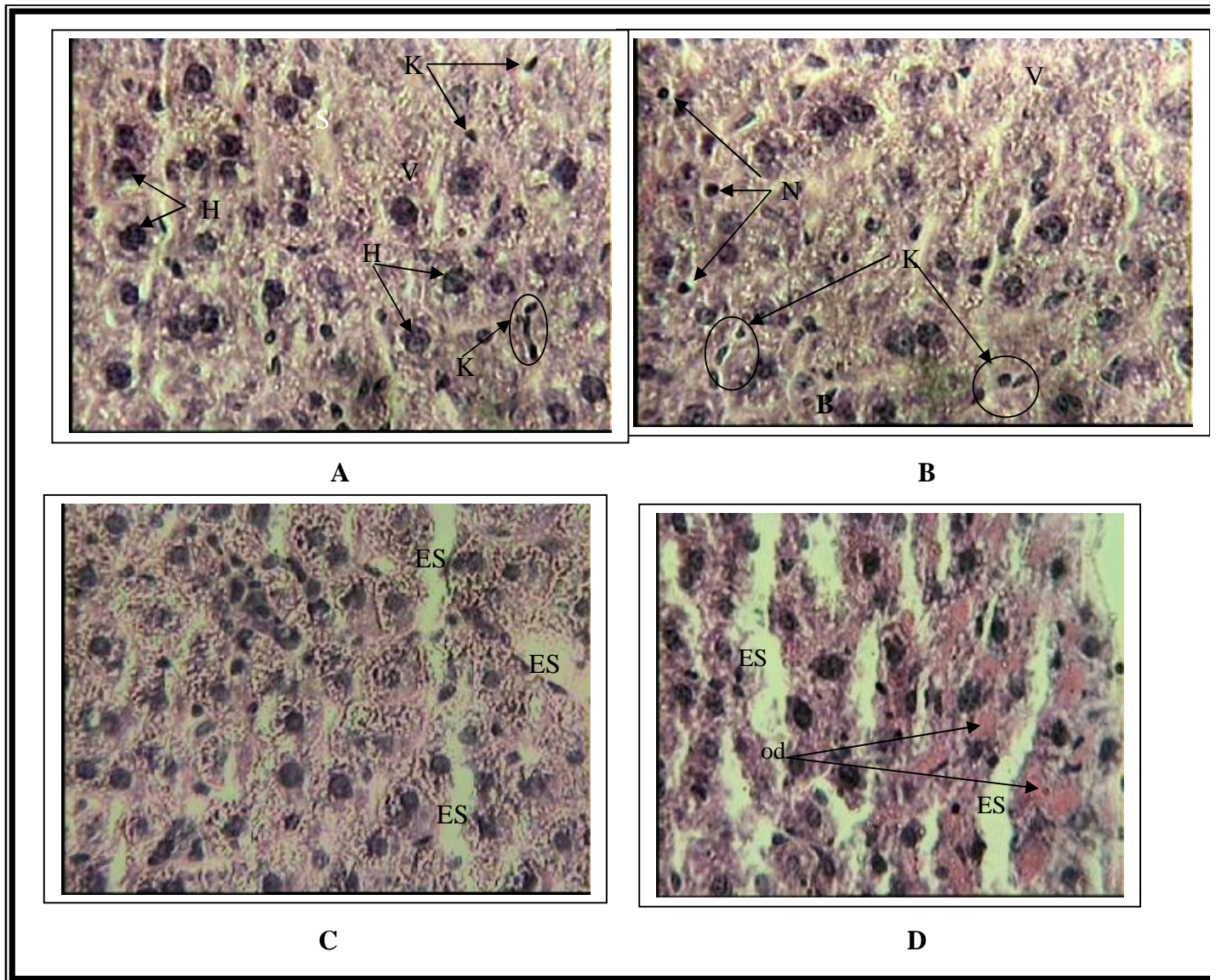


Figure-2:- Hepatic tissue of mice treated with PGF_2a showed:

A: Hypertrophic hepatic cells with irregular nuclear envelope(H), vacuolation of cytoplasm (V), increasing of Kupffer cell number(K) .

B: Necrotic hepatocytes (N).

C,D : Enlarged sinusoids (ES).

(HE: 600X)

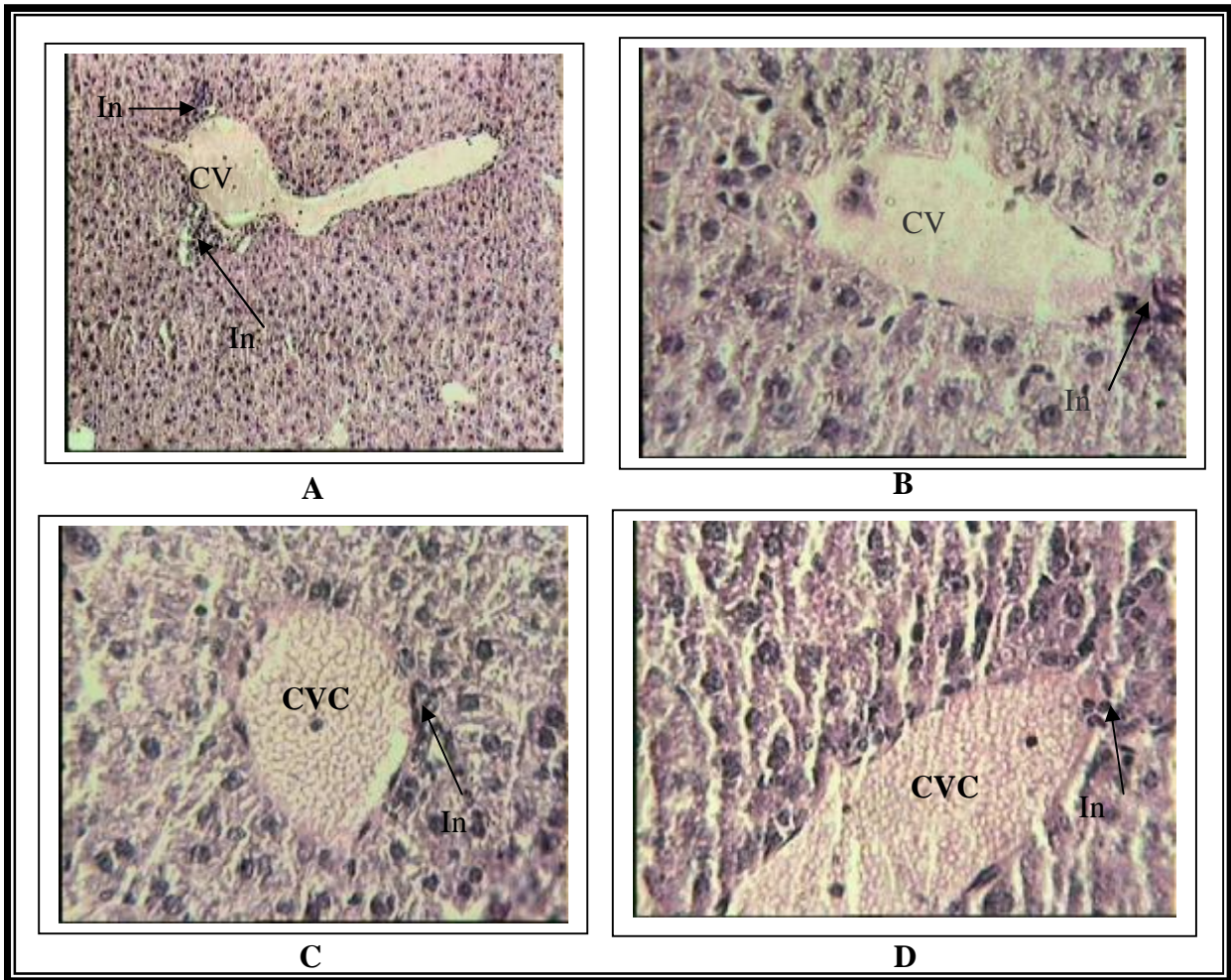


Figure-3:- Hepatic tissue of mice treated with PGF_2a showed:

A,B :Infiltration of Inflammatory cells (In) at periphery of central vein(CV) . (HE:A 150X, B 600X)

C,D : Central vein congestion (CVC) (HE: 600X)

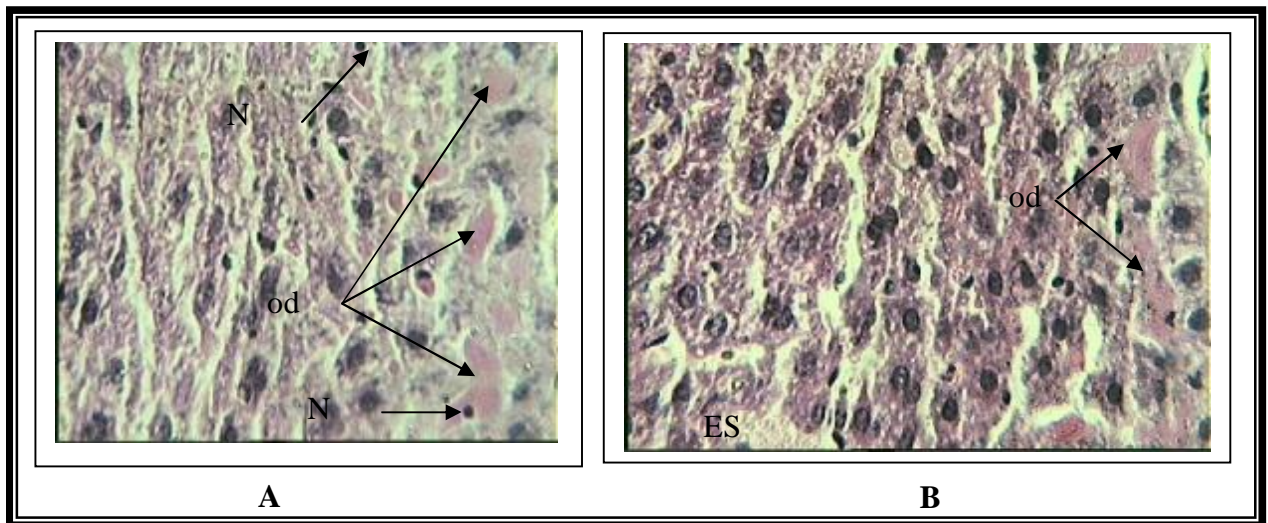


Figure-4- Hepatic tissue of mice treated with PGF_2a showed odema (od) , some enlarged sinusoids (ES) and necrotic hepatocytes (N). (HE: 600X).

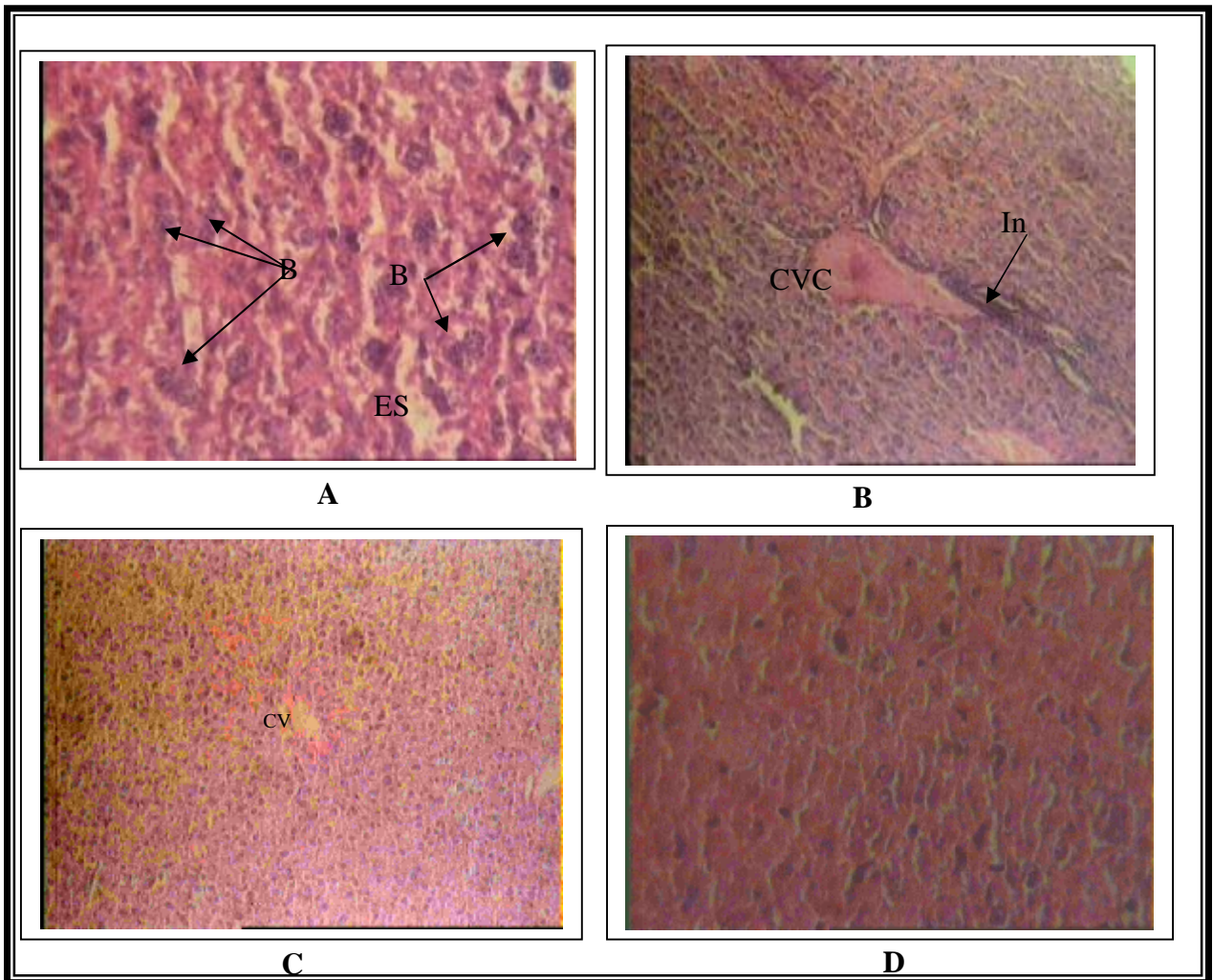


Figure-5:-Hepatic tissue of mice treated with fenugreek seeds and PGF_2a injection showed:

A: Binucleated hepatocytes (B) , some enlarged sinusoids (ES). (HE: 600X).

B: Central vein congestion (CVC) and Infiltration of Inflammatory cells (In) (HE: 150X) .

C,D: Normal architecture of many parts of hepatic lobules , CV: central vein (HE:C150 X,D600X)

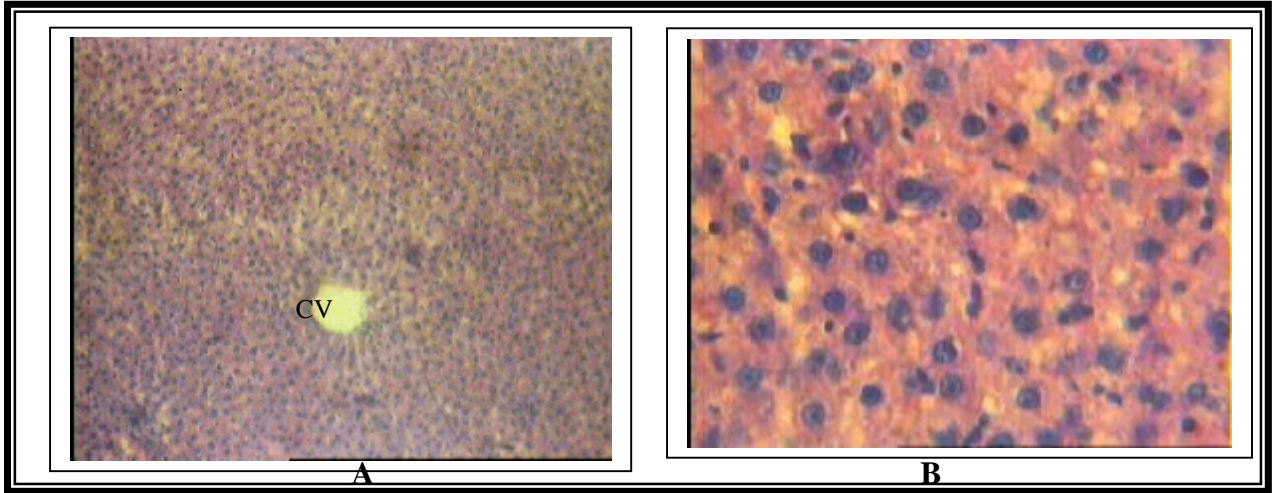


Figure-6:- Hepatic tissue of mice treated with normal physiological saline (control group) showed normal architecture of hepatic lobules and hepatocytes , CV: central vein (HE: A150 X , B 600X)